(54) Title: SYNTHETIC IgE MEMBRANE ANCHOR PEPTIDE IMMUNOGENS FOR THE TREATMENT OF ALLERGY

(57) Abstract

The present invention relates to a method for eliciting the production in healthy mammals, including humans, of high titer antibodies specific for sites on the extracellular segment of the anchor domain of the membrane-bound ε heavy chain of B cell-expressed human IgE by the use of a composition comprising a synthetic peptide immunogen containing extracellular membrane anchor sites, to reduce IgE-secreting B leukocytes and allergen-induced IgE production. It also relates to the use of optimally designed, carrier protein free, IgE ε-chain related immunogens as key components in a synthetic vaccine to provide an immunotherapy for the treatment of allergy. The subject peptides contain immune stimulator sequences, including a tandemly linked helper T cell epitope, to aid in stimulating the immune response towards the mIgE membrane anchor domain.

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SYNTHETIC IgE MEMBRANE ANCHOR PEPTIDE IMMUNOGENS FOR THE TREATMENT OF ALLERGY

FIELD OF THE INVENTION

The present invention relates to the use of a composition of a synthetic peptide immunogen comprising a target antigenic site and a helper T cell epitope covalently linked in a linear tandem form. More particularly, the present invention relates to the use of such a composition to elicit the production in healthy mammals, including humans, of high titer antibodies specific to sites on the ε heavy chain of B cell-expressed membrane-bound human IgE, i.e., sites on the extracellular segment of the anchor domain of membrane-bound human ε-chain and to the use of such a composition as a vaccine to provide an immunotherapy for the treatment of allergy.

BACKGROUND OF THE INVENTION

Immunotherapy for the prevention of IgE-mediated allergic responses such as asthma and hay fever, as known and practiced since early in this century, has involved desensitization or hyposensitization by administering a gradually increasing amount of an allergen to a patient to reduce the effects of subsequent exposure to that allergen[1]. Limitations to such an allergen-based immunotherapy include difficulties in identifying the allergen involved and the adverse reactions frequently caused by the use of the identified allergen[2]. Other treatments for the relief of allergies employ therapeutic compounds to block the cascade of cellular events that is responsible for allergic reactions. These compounds include anti-histamines, decongestants, β_2 agonists, and corticosteroids. Anti-histamines, decongestants, and β_2 agonists act on events downstream of IgE in the allergic cascade, making them palliative remedies which address allergic symptoms rather than preventative treatments which must act on events closer to the initiation of IgE-

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mediated allergic reactions. These palliative remedies
provide relief that is short term and partial, frequently
accompanied by adverse side effects. For example, anti-
histamines may cause restlessness or sedation, and β_2
5   agonists are sometimes associated with increased morbidity
in asthmatic patients.

In contrast to anti-histamines, decongestants
and β_2 agonists, corticosteroids are powerful
immunosuppressants and are highly efficacious for the
10   treatment of allergic symptoms. However, they produce
adverse hormonal activities and may cause an undesirably
broad immunosuppression. To avoid the shortcomings of
these therapeutic compounds, it would be desirable to
prevent allergic responses at the level of IgE, with a
15   means of suppression selectively targeted to IgE. This
may be accomplished by suppressing IgE synthesis directly
or indirectly. Indirect suppression can be accomplished
by desensitization or by inhibition of IL-4 and other T
cell-produced mediators of IgE synthesis[3]. Direct
20   suppression, as suggested by Chang et al.[4], can be
accomplished by specifically targeting IgE-producing B
cells with selective antibodies.

Chang et al.[4-8] and others[9] have studied human
ε-chains and corresponding antibodies, as well as the
25   genes and mRNAs by which the ε-chains are encoded. They
have elucidated the molecular basis for the expression of
two types of IgE: the secreted and membrane-bound forms by
B cells committed to IgE synthesis. The membrane-bound
form of IgE (mIgE) may be distinguished from the secreted
30   form by an additional membrane anchoring domain that
extends from the C-terminus of the heavy chains and is
contiguous with the CH4 constant domain of IgE. The
membrane-bound form is distinctive to the surface of B
cells committed to IgE synthesis. By targeting such cells
35   with antibodies specific for the exposed extracellular
portion of that anchor domain, such cells may be

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eliminated or inactivated. The mechanisms for
elimination of IgE-secreting cells by such antibodies can
be through antibody-dependent cellular cytotoxicity (ADCC)
and complement-mediated cytolysis[6,7]. The reduction of
circulating IgE and IgE-expressing cells by anti-IgE
antibodies has been demonstrated *in vivo* in mice[10], and by
the inhibition *in vivo* of passive cutaneous anaphylaxis in
a rat model. It has also been demonstrated in human IgE-
secreting cell lines where anti-IgE was shown to lead to
reductions in cell growth, decreased IgE accumulation and
cytolysis in complement-mediated and ADCC-mediated
cytolysis assays[11]. By determining the nucleotide
sequences of pertinent segments in human genomic DNA and
in mRNA from human mIgE-expressing B cells, amino acid
sequences for the extracellular portion of the anchor
domain were predicted. The presence and specificity of
these sites as well as their accessibility to antibodies
were confirmed with specific antibodies[4,8]. These
polyclonal and monoclonal antibodies were anti-peptide
antibodies derived from immunizations with ε chain-related
"peptide-carrier protein conjugate"[4,8]. The carrier
protein was Keyhole Limpet Hemocyanin (KLH) known to be
useful for its capability of stimulating antibody
responses to a target peptide. This approach can be used
to demonstrate the utility of the mIgE-specific peptide
immunogen for the immunotherapy of allergic disease either
by passive immunization with monoclonal antibodies, or by
active immunization[6,7].

The feasibility of using such a peptide-KLH
vaccine to provide immunotherapy to patients with IgE-
mediated sensitivities has been reported by Stanworth et
al. using a different ε-chain peptide[12,13]. A rabbit anti-
peptide serum, selected from bleeds obtained from multiple
immunizations which produced better-than-average anti-
peptide titer, reduced induced histamine release from rat
peritoneal mast cells in a titer-dependent fashion. This

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inhibitory activity was further confirmed by *in vivo* tests in a rat passive cutaneous anaphylaxis (PCA) model. The effect of this rabbit anti-peptide serum on anaphylaxis was assessed by measurement of the area of blueing and by an estimate of the color intensity when given to rats which had been previously sensitized by multiple allergen application prior to anaphylactic challenge with allergen. These results were preliminary indications for the feasibility of using peptide-based vaccines for the treatment of allergy. However, this peptide-conjugate strategy has met with considerable difficulties. For example, it has been found that the resultant clones and antisera raised by such conjugates contain more antibodies directed at the epitopes on the protein carrier, KLH, than to the target-peptide[14]. Other major deficiencies of protein carrier conjugate vaccines include: less-than-optimal immune stimulatory capability, manufacturing difficulties stemming from the poorly defined composition of the carrier protein, and the non-uniformity of the conjugation reaction.

Those skilled in the art usually use a conjugated protein carrier for synthetic peptide immunogens because small peptides are poor immunogens. In order to be rendered immunogenic, these peptides are usually conjugated to large carrier proteins chemically or by gene fusion. These processes, however, can produce unpredictable conformational changes in peptides. Moreover, the immune response is frequently misdirected to the immunodominant carrier. Consequently, the development of a potent vaccine that provides long-lasting protection from allergies awaits further immunogen design. Careful explorations into the design of "optimal immunogens," including full validation by the results of extensive experimental trials, still remain to be accomplished.

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LIST OF REFERENCES

1.   Noon. "Prophylactic inoculation against hay
      fever." Lancet, i:1572-1573 (1911).

2.   World Health Organization and International
      Union of Immunological Societies Working Group
      Report: Current status of allergen
      immunotherapy. Lancet, i:259-261 (1989).

3.   Pène et al. Proc. Natl. Acad. Sci. USA.,
      85:6880-6884   (1988).

4.   Davis, Gosset, and Chang. Bio/Technol., 9:53-56
      (1991).

5.   Sun, Liou, Sun, Gosset, et al. J. Immunol.,
      146:199-205 (1991).

6.   Chang. Antigenic epitopes of IgE present on B
      cell but not basophil surface. U.S. patent no.
      5,091,313 (1992).

7.   Chang. Newly identified human epsilon
      immunoglobulin peptides and related products.
      U.S. patent no. 5,274,075 (1993).

8.   Peng, Davis, Sun, et al. J. Immunol., 148:129-
      136 (1992).

9.   Saxon, Kurbe-Leamer, et al. J. Immunol.,
      147:4000-4006 (1991).

10.   Haba and Nisonoff. Proc. Natl. Acad. Sci. USA.,
      87:3363-3367 (1990).

11.   Davis, Gosset, Pinkston, Liou, et al. Springer
      Semin. Immunopath., 15:51-73 (1993).

12.   Stanworth, Jones, Lewin, and Nayyar. Lancet,
      336:1279-1281 (1990).

13.   Stanworth, Lewin, Nayyar, and Jones.
      Immunoreactive peptides and antibodies and their
      use in anti-allergy treatment. EPO 403 312 A1
      (1990).

14.   Stanworth, and Burt. Mol. Immunol., 23:1231-1235
      (1986).

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15. Brett, et al. _Eur. J. Immunol._, 23:1608-1614 (1993).

16. Weismuller, et al. _Int. J. Peptide Res._, 40:255-260 (1992).

17. Celis, et al. _J. Immunol._, 140:1808-1815 (1988).

18. Demotz, et al. _J. Immunol._, 142:394-402 (1989).

19. Chong ,et al. _Infect. Immun._, 60:4640-4647 (1992).

20. Grant, ed. _Synthetic Peptides: A User's Guide_, W.H.Freeman & Co., New York, NY, (1992) pp. 382.

21. O'Hagan, et al. _Vaccine_, 9:768-771 (1991).

22. Eldridge, et al. _Molec. Immunol._, 28:287-294 (1991).

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OBJECTS OF THE INVENTION

It is an objective of the present invention to
employ a group of membrane-bound IgE (mIgE) ε-chain
peptide immunogens chemically synthesized linearly in
tandem with T helper epitope peptides, which when
introduced to mammals, including humans, can elicit high
titer antibodies to exposed peptide sites of the membrane
anchoring domain of human mIgE.

Another objective is to design optimal peptide
immunogens, with specific amino acid sequences taken from
the human mIgE heavy chain membrane anchoring domain (SEQ
ID NOS:1,2) and attached to peptides containing
promiscuous human helper T cell epitopes in specified
orientations which, when introduced into mammals,
including humans, will stimulate production of efficacious
antibodies to the sites on human mIgE anchor domain.
These antibodies may result in the reduction in IgE-
producing B lymphocytes and thereby attenuate allergen-
induced IgE production, constrain mast cell activation by
IgE-allergen complexes, reduce the consequent release of
chemical mediators such as histamines responsible for
allergic symptoms and depress IgE-mediated passive
cutaneous anaphylaxis (PCA). The end result is expected
to be the reduction of allergic symptoms.

Another objective is to develop an effective
mIgE ε-chain peptide-based vaccine, employing compositions
containing such linear peptide immunogens, so as to
provide immunotherapy for the treatment of allergic
reactions.

SUMMARY OF THE INVENTION

According to the present invention, a series of
linearly arranged synthetic peptides which contain either
of two peptide sequences corresponding to sites on the
exposed portion of the membrane anchoring domain of human
mIgE (SEQ ID NOS:1,2) or their immunogenic analogs thereof

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together with a portion of a helper T-cell epitope (Th epitope) are made by solid phase synthesis. Compositions of the invention are used to immunize healthy mammals, e.g. rats and humans for the production of high titer antisera that is specific for the mIgE anchor membrane sites (SEQ ID NOS:1,2) and free of irrelevant antibodies.

According to the present invention, vaccines containing the synthetic peptide compositions as the key immunogen may also be prepared with an immunologically effective amount of linear synthetic peptide in the presence of a proper adjuvant and/or delivery vehicle. It is expected that such vaccine compositions will elicit a more focused anti-IgE peptide response than those of the peptide-carrier protein conjugates currently used by Chang et al.[4,6-8], thus providing better immunotherapy for the treatment of allergy.

DETAILED DESCRIPTION OF THE INVENTION

This invention is directed to the use of a novel group of peptide-based immunogens for the generation of high titer antibodies to mIgE anchor membrane sites (SEQ ID NOS:1,2) on human IgE ε heavy chain in healthy mammals, including humans, ultimately for the treatment of IgE-mediated allergic diseases.

Table I shows the arrangement and the amino acid sequence of the Membrane Anchor Domain of the ε heavy chain of human membrane-bound IgE (mIgE), as deduced from the nucleic acid sequence of the predominant species of mRNA that codes for membrane-bound ε chain[7,8]. The regions on the ε chain sequence of the peptides used as the target immunogens of the invention are underlined: a single underline for SEQ ID NO:1, and a double underline for SEQ ID NO:2.

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TABLE I

CH4 Domain | Membrane Anchor Domain/extracellular segment O...
ValAsnPro | GlyLeuAlaGlyGlySerAlaGlnSerGlnArgAlaProAspArgVal

LeuCysHisSerGlyGlnGlnGlnGlyLeuProArgAlaAlaGlyGlySerValProHisPro

                                                    /extracellular
ArgCysHisCysGlyAlaGlyArgAlaAspTrpProGlyProProGluLeuAspValCysVal

segment 1   /transmembrane anchor segment
GluGluAlaGluGlyGluAlaProTrpThrTrpThrGlyLeuCysIlePheAlaAlaLeuPhe

                                        /cytoplasmic anchor segment
LeuLeuSerValSerTyrSerAlaAlaLeuThrLeuLeuMetValGlnArgPheLeuSerAla

ThrArgGlnGlyArgProGlnThrSerLeuAspTyrThrAsnValLeuGlnProHisAla

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It is generally accepted by people skilled in the art that allergic symptoms, the immediate result of IgE-dependent hypersensitivities, are caused by the chemical mediators released by mast cells and basophils. This release is triggered when secretory IgE ligated to receptors on a mast cell or basophil is bound by the allergen for which the receptor-bound IgE is specific. The triggering event is actuated after the allergen binds to the the Fab' portion of the surface-bound IgE in an antigen-antibody type interaction. The allergen/antibody binding crosslinks the bivalent surface-bound IgE and induces conformational changes in the distal Fc region of IgE, the region of IgE in direct contact with a high affinity Fc receptor on the mast/basophil cell surface as well as receptor site(s) on the cell surface. By a mechanism as yet not precisely known, the conformational changes activate the cell-IgE-allergen complex with the resultant release from the cell of chemical mediators, including histamine, inducing allergic symptoms and the further secretion of IgE. The secretory IgE which mediates the allergic reaction is produced by terminally differentiated B cells in response to allergen.

Besides secreting the circulatory IgE which becomes bound to mast cells and basophils, B cells committed to IgE synthesis also display membrane-bound IgE (mIgE) on their surface. The mIgE molecules are allergen receptors and are believed to play regulatory roles in the maturation of the B cells, and in activation of the B cells by allergen-specific T cells. The mIgE is distinguishable from the secreted IgE by a membrane-anchoring segment which extends from the C-terminus of the heavy chains which serves to attach the mIgE to the cell membrane. By determining the nucleotide sequences of pertinent segments in human genomic DNA and in mRNA from human mIgE-expressing B cells, amino acid sequences for two immunogenic sites on the extracellular portion of the

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anchor domain were deduced (SEQ ID NOS:1,2). These sites
are present on alternative forms of mIgE that result from
different mRNA splicing events. Both sites are present on
the predominant species of membrane-bound ε-chain, in the
orientation shown in Table I. The presence and
specificity of these sites as well as their accessibility
to antibodies were confirmed with specific antibodies[4,8].
The presence of anti-IgE antibodies directed against such
specific mIgE sites, through either active or passive
immunization, can lead to the reduction in the numbers of
IgE-producing B cells and a concomitant reduction in
circulating IgE, possibly through the lytic removal of the
IgE-expressing cells[10,11]. Moreover, it is desirable to
target anti-IgE antibodies to the membrane-anchor domain
because the domain is a surface marker specific to IgE-
expressing cells. This target site is not available on
secreted IgE. Thus, such anti-mIgE cannot bind and
crosslink IgE bound to receptors on mast cells and
basophils, and cannot by itself induce histamine release.
The removal of IgE-expressing cells in hosts suffering
from allergic reactions may result in the down-regulation
of IgE production and have a therapeutic outcome.

Such interventions employed in the treatment of
allergy through the use of specific anti-IgE antibodies,
i.e. a kind of immunotherapy, can be achieved either
passively, through prophylactic treatment with specific
"site-directed" antibodies to IgE, or, more preferably,
actively, by providing the host with a vaccine comprised
of site-directed peptide immunogens, to elicit the
production by the host of its own site-directed anti-IgE
antibodies. It is believed that active immunization will
provide a more effective and longer lasting form of
protection.

The sites on the extracellular segment of the
membrane anchor domain (SEQ ID NOS:1,2), arranged on
membrane-bound IgE as shown in Table I, have been

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confirmed as immunogenic sites that are acessible to
antibodies through the cross-reactivities of the surface
of IgE-secreting cells to antibodies that had been
generated by immunizing animals with mIgE anchor membrane
peptides coupled to a carrier protein, keyhole limpet
hemocyanin (KLH)[4,8].

A major deficiency of these prototype "mIgE
peptide" vaccines are their weak immunogenicities, an
inherent problem associated with almost all self-antigens.
In the present invention, specific examples are provided
for the linkage of synthetic immunostimulatory elements to
the mIgE peptides (SEQ ID NOS:1,2) in specified
orientations such that potent antibodies directed to these
sites on mIgE can be broadly generated in a genetically
diverse host population. In turn, these antibodies may
lead to reduction of IgE-expressing cells, reduced levels
of circulating IgE, and diminished IgE-mediated responses,
thus resulting in an effective treatment for the
prevention of IgE-mediated allergic diseases.

The peptide immunogens of the current invention,
while being substantially incapable of mediating non-
cytolytic histamine release, are capable of eliciting
antibodies with serological cross-reactivity with the
target amino acid sequences of the extracellular region of
the human mIgE membrane anchor domain (SEQ ID NOS:1,2).

The initial dose, e.g. 0.2-2.5 mg; preferably 1
mg, of immunogen is to be administered by injection,
preferably intramuscularly, followed by repeat (booster)
doses. Dosage will depend on the age, weight and general
health of the patient as is well known in the vaccine and
therapeutic arts.

While there is no particular limitation to
species of mammals provided for the preparation of
antibodies, it is generally preferred to use mice,
rabbits, guinea pigs, pigs, goats, rats or sheep, etc. as
the hosts.

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For active immunization, the term "immunogen" referred to herein relates to a synthetic peptide which is capable of inducing antibodies against the mIgE membrane anchor domain (SEQ ID NOS:1,2), leading to suppression of IgE-mediated basophil and mast cell degranulation. The immunogen of the present invention includes linear synthetic peptides which contain promiscuous helper T cell epitopes (Th epitopes). The Th peptides are covalently attached to the mIgE membrane anchor domain peptide (SEQ ID NOS:1,2), with a spacer, so as to be adjacent to either the N- or C-terminus of the membrane anchor peptides, in order to evoke efficient antibody responses. The immunogen may also comprise an immune stimulatory amino acid sequence corresponding to a domain of an invasin protein from the bacteria *Yersinia* spp[15]. The invasin domain is attached through a spacer to a Th peptide.

The immunogen of the present invention minimizes the generation of irrelevant antibodies to elicit a more focused immune response to the "target sequences", the desired reactivity to mIgE membrane anchor sites (SEQ ID NOS:1,2), without producing undesirable side effects which may complicate the immunotherapy process for the treatment of allergy. However, when the desired target sequence is short, such as the 26 and 17 amino acid mIgE peptides (SEQ ID NOS:1,2) of the present invention, one faces other challenges because such short peptide antigens are usually T cell-dependent antigens, i.e. the presence of a T helper epitope is required to render such antigens immunogenic. Much effort had to be expended in the design of linear synthetic immunogens containing the short mIgE anchor membrane peptides (SEQ ID NOS:1,2) to provide for functional T-cell epitopes.

The peptides of this invention are represented by the formulas

$$(A)_n-(Th)_m-(B)_o-(mIgE\ peptide)$$
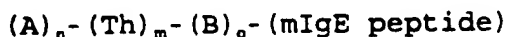
wherein:

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A is an amino acid, α-NH₂, a fatty acid or a derivative thereof, or an invasin domain;

B is an amino acid;

Th is a helper T cell epitope or an immune enhancing analog or segment thereof; mIgE peptide is:

Gly-Leu-Ala-Gly-Gly-Ser-Ala-Gln-Ser-Gln-Arg-Ala-Pro-Asp-Arg-Val-Leu-Cys-His-Ser-Gly-Gln-Gln-Gln-Gly-Leu   (SEQ ID NO:1);

or

Pro-Glu-Leu-Asp-Val-Cys-Val-Glu-Glu-Ala-Glu-Gly-Glu-Ala-Pro-Trp-Thr (SEQ ID NO:2);

or an immunogenic analog thereof;

n is from 1 to about 10;

m is from 1 to about 4; and

o is from 0 to about 10.

The peptide immunogen of the present invention comprises from about 20 to about 100 amino acid residues, preferably from about 20 to about 50 amino acid residues and more preferably from about 20 to about 35 amino acid residues.

When A is an amino acid, it can be any non-naturally occurring or any naturally occurring amino acid. Non-naturally occurring amino acids include, but are not limited to, ß-alanine, ornithine, norleucine, norvaline, hydroxyproline, thyroxine, γ-amino butyric acid, homoserine, citrulline and the like. Naturally-occurring amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. Moreover, when m is greater than one, and two or more of the A groups are amino acids, then each amino acid may be independently the same or different.

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When A is a fatty acid, such as stearic acid,
palmitic acid or a fatty acid derivative, such as
tripalmitoyl cysteine (Pam₃Cys), it acts as an adjuvant by
enhancing the immune stimulating properties of the Th
epitope[16]. The fatty acid moiety or its derivative is
preferably located at the amino terminus of the mIgE
peptide. When A is a fatty acid or its derivative, the
peptide immuogen comprises 2 or 3 additional A moieties
which are amino acids. As used herein, the fatty acid is
selected from the group with a hydrocarbon chain of 8 to
24 carbon atoms. The hydrocarbon chain can be saturated
or unsaturated.

When A is an invasin domain, it is an immune
stimulatory epitope from the invasin protein of a *Yersinia*
species. This immune stimulatory property results from
the capability of this invasin domain to interact with the
ß1 integrin molecules present on T cells, particularly
activated immune or memory T cells. The specific sequence
for an invasin domain found to interact with the $\beta 1$
integrins has been decribed by Brett et al [15],
incorporated herein by reference. In a preferred
embodiment, the invasin domain (Inv) for linkage to a
promiscuous Th epitope has the sequence:

Thr-Ala-Lys-Ser-Lys-Lys-Phe-Pro-Ser-Tyr-Thr-Ala-
Thr-Tyr-Gln-Phe   (SEQ ID NO:3)
or is an immune stimulatory analog thereof from the
corresponding region in another *Yersinia* species invasin
protein. Such analogs thus may contain substitutions,
deletions or insertions of amino acid residues to
accommodate strain to strain variation, provided that the
analogs retain immune stimulatory properties.

In one embodiment, n is 1 and A is α-NH₂. In
another embodiment, n is 4 and A is α-NH₂, an invasin
domain (Inv), glycine and glycine, in that order.

B is a spacer and is an amino acid which can be
naturally occurring or the non-naturally occurring amino

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acids as described above. Each B is independently the
same or different. In addition, the amino acids of B can
form a flexible hinge, or spacer, to enhance the immune
response to the Th epitope and mIgE peptides and their
immunogenic analogs thereof. Examples of sequences
encoding flexible hinges are found in the immunoglobulin
heavy chain hinge region. Flexible hinge sequences are
often proline rich. One particularly useful flexible
hinge is provided by the sequence Pro-Pro-Xaa-Pro-Xaa-Pro
(SEQ ID NO:4), where Xaa is any amino acid, and preferably
aspartic acid.

        Immunogenicity can also be improved through the
addition of spacer residues (e.g. Gly-Gly) between the
promiscuous Th epitope and the mIgE membrane anchor
peptides (SEQ ID NOS:1,2) and immunogenic analogs thereof.
In addition to physically separating the Th epitope from
the B cell epitope, i.e., the mIgE membrane anchor
peptides (SEQ ID NOS:1,2) and immunological analogs
thereof, the spacer glycine residues can disrupt any
artifactual secondary structures created by the joining of
the Th epitope with the the mIgE membrane anchor peptides
(SEQ ID NOS:1,2) and immunogenic analogs thereof and
thereby eliminate interference between the T and/or B cell
responses. The conformational separation between the
helper cell and the antibody eliciting domains thus
permits more efficient interactions between the presented
immunogen and the appropriate Th and B cells.

        Th is a sequence of amino acids (natural or non-
natural amino acids) that comprises a Th epitope. A Th
epitope can consist of a continuous or discontinuous
epitope. Hence not every amino acid of Th is necessarily
part of the epitope. Accordingly, Th epitopes, including
analogs and segments of Th epitopes, are capable of
enhancing or stimulating an immune response to the mIgE
membrane anchor peptides (SEQ ID NOS:1,2) and
immunological analogs thereof. Th epitopes that are

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immunodominant and promiscuous are highly and broadly reactive in animal and human populations with widely divergent MHC types[17-19]. The Th domain of the subject peptides has from about 10 to about 50 amino acids and preferably from about 10 to about 30 amino acids. When multiple Th epitopes are present (i.e. m ≥ 2), then each Th epitope is independently the same or different.

Th epitope analogs include substitutions, additions, deletions and insertions of from one to about 10 amino acid residues in the Th epitope. Th segments are contiguous portions of a Th epitope that are sufficient to enhance or stimulate an immune response to the mIgE membrane anchor peptides (SEQ ID NOS:1,2) and immunological analogs thereof.

Th epitopes of the present invention include hepatitis B surface and core antigen helper T cell epitopes (HB$_s$Th and HB$_c$Th), pertussis toxin helper T cell epitopes (PT Th), tetanus toxin helper T cell epitopes (TT Th), measles virus F protein helper T cell epitopes (MV$_F$ Th), *Chlamydia trachomatis* major outer membrane protein helper T cell epitopes (CT Th), diphtheria toxin helper T cell epitopes (DT Th), *Plasmodium falciparum* circumsporozoite helper T cell epitopes (PF Th), *Schistosoma mansoni* triose phosphate isomerase helper T cell epitopes (SM Th), *Escherichia coli* TraT helper T cell epitopes (TraT Th) and immune-enhancing analogs and segments of any of these Th epitopes. Examples of Th epitope sequences are provided below as Table II:

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TABLE II

HB, Th: Phe-Phe-Leu-Leu-Thr-Arg-Ile-Leu-Thr-Ile-Pro-Gln-
          Ser-Leu-Asp   (SEQ ID NO:5)

PT₁ Th: Lys-Lys-Leu-Arg-Arg-Leu-Leu-Tyr-Met-Ile-Tyr-Met-
          Ser-Gly-Leu-Ala-Val-Arg-Val-His-Val-Ser-Lys-Glu-
          Glu-Gln-Tyr-Tyr-Asp-Tyr   (SEQ ID NO:6)

TT₁ Th: Lys-Lys-Gln-Tyr-Ile-Lys-Ala-Asn-Ser-Lys-Phe-Ile-
          Gly-Ile-Thr-Glu-Leu   (SEQ ID NO:7)

TT₂ Th: Lys-Lys-Phe-Asn-Asn-Phe-Thr-Val-Ser-Phe-Trp-Leu-
          Arg-Val-Pro-Lys-Val-Ser-Ala-Ser-His-Leu
                                              (SEQ ID NO:8)

PT₁ₐ Th: Tyr-Met-Ser-Gly-Leu-Ala-Val-Arg-Val-His-Val-Ser-
          Lys-Glu-Glu   (SEQ ID NO:9)

TT₃ Th: Tyr-Asp-Pro-Asn-Tyr-Leu-Arg-Thr-Asp-Ser-Asp-Lys-
          Asp-Arg-Phe-Leu-Gln-Thr-Met-Val-Lys-Leu-Phe-Asn-
          Arg-Ile-Lys   (SEQ ID NO:10)

PT₂ Th: Gly-Ala-Tyr-Ala-Arg-Cys-Pro-Asn-Gly-Thr-Arg-Ala-
          Leu-Thr-Val-Ala-Glu-Leu-Arg-Gly-Asn-Ala-Glu-Leu
                                              (SEQ ID NO:11)

MV_F1 Th: Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-
          Glu-Gly-Val   (SEQ ID NO:12)

HB_c Th: Val-Ser-Phe-Gly-Val-Trp-Ile-Arg-Thr-Pro-Pro-Ala-
          Tyr-Arg-Pro-Pro-Asn-Ala-Pro-Ile-Leu
                                              (SEQ ID NO:13)

MV_F2 Th: Gly-Ile-Leu-Glu-Ser-Arg-Gly-Ile-Lys-Ala-Arg-Ile-
          Thr-His-Val-Asp-Thr-Glu-Ser-Tyr   (SEQ ID NO:14)

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TT₄ Th: Trp-Val-Arg-Asp-Ile-Ile-Asp-Asp-Phe-Thr-Asn-Glu-
           Ser-Ser-Gln-Lys-Thr   (SEQ ID NO:15)

TT₅ Th: Asp-Val-Ser-Thr-Ile-Val-Pro-Tyr-Ile-Gly-Pro-Ala-
           Leu-Asn-Ile-Val   (SEQ ID NO:16)

CT Th: Ala-Leu-Asn-Ile-Trp-Asp-Arg-Phe-Asp-Val-Phe-Cys-
           Thr-Leu-Gly-Ala-Thr-Thr-Gly-Tyr-Leu-Lys-Gly-Asn-
           Ser   (SEQ ID NO:17)

DT₁ Th: Asp-Ser-Glu-Thr-Ala-Asp-Asn-Leu-Glu-Lys-Thr-Val-
           Ala-Ala-Leu-Ser-Ile-Leu-Pro-Gly-Ile-Gly-Cys
                                           (SEQ ID NO:18)

DT₂ Th: Glu-Glu-Ile-Val-Ala-Gln-Ser-Ile-Ala-Leu-Ser-Ser-
           Leu-Met-Val-Ala-Gln-Ala-Ile-Pro-Leu-Val-Gly-Glu-
           Leu-Val-Asp-Ile-Gly-Phe-Ala-Ala-Thr-Asn-Phe-Val-
           Glu-Ser-Cys   (SEQ ID NO:19)

PF Th: Asp-Ile-Glu-Lys-Lys-Ile-Ala-Lys-Met-Glu-Lys-Ala-
           Ser-Ser-Val-Phe-Asn-Val-Val-Asn-Ser
                                           (SEQ ID NO:20)

SM Th: Lys-Trp-Phe-Lys-Thr-Asn-Ala-Pro-Asn-Gly-Val-Asp-
           Glu-Lys-Ile-Arg-Ile   (SEQ ID NO:21)

TraT₁ Th: Gly-Leu-Gln-Gly-Lys-His-Ala-Asp-Ala-Val-Lys-Ala-
           Lys-Gly   (SEQ ID NO:22)

TraT₂ Th: Gly-Leu-Ala-Ala-Gly-Leu-Val-Gly-Met-Ala-Ala-Asp-
           Ala-Met-Val-Glu-Asp-Val-Asn   (SEQ ID NO:23)

TraT₃ Th: Ser-Thr-Glu-Thr-Gly-Asn-Gln-His-His-Tyr-Gln-Thr-
           Arg-Val-Val-Ser-Asn-Ala-Asn-Lys (SEQ ID NO:24)

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The linear synthetic peptides of this invention, as described by the formulas $(A)_n-(Th)_m-(B)_o-$(mIgE peptide) or (mIgE peptide)$-(B)_o-(Th)_m-(A)_n$, have the Th epitope covalently attached through spacer B to the N terminus of either of the the mIgE membrane anchor peptides (SEQ ID NOS:1,2) and immunogenic analogs thereof. In a preferred embodiment the Th epitope is HB$_1$ Th, PT$_1$ Th, PT$_2$ Th, TT$_1$ Th, TT$_3$ Th, or MV$_{F1}$ Th.

The sequence of the mIgE membrane anchor peptide comprises:

                    Gly-Leu-Ala-Gly-Gly-Ser-Ala-Gln-Ser-Gln-
                    Arg-Ala-Pro-Asp-Arg-Val-Leu-Cys-His-Ser-
                    Gly-Gln-Gln-Gln-Gly-Leu   (SEQ ID NO:1);
                                    or
                    Pro-Glu-Leu-Asp-Val-Cys-Val-Glu-Glu-Ala-
                    Glu-Gly-Glu-Ala-Pro-Trp-Thr   (SEQ ID NO:2).

Immunogenic peptide analogs of the mIgE anchor peptides (SEQ ID NOS:1,2) according to the invention, may further comprise substitutions, additions, deletions, or insertions of from one to about four amino acid residues provided that the peptide analogs are capable of eliciting immune responses crossreactive with the mIgE membrane anchor peptides (SEQ ID NOS:1,2). The substitutions, additions, and insertions can be accomplished with natural or non-natural amino acids as defined herein.

Accordingly, preferred peptide immunogens of this invention are the linear synthetic peptides containing the mIgE membrane anchor peptides (SEQ ID NOS:1,2) or immunological analogs thereof and Th. The more preferred peptide immunogens are those linear constructs containing the mIgE membrane anchor peptides (SEQ ID NOS:1,2) or immunogenic analogs thereof; a spacer (e.g Gly-Gly); a Th epitope selected from the group consisting of HB$_1$ Th, PT$_1$ Th, PT$_2$ Th, TT$_1$ Th, TT$_3$ Th, and MV$_{F1}$ Th (SEQ ID NOS:5,6,11,7,10,12, respectively); and, optionally, an Inv domain (SEQ ID NO:3) or analog thereof.

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The peptide immunogens of this invention can be made by chemical synthesis methods which are well known to the ordinarily skilled artisan. See, for example, Grant, ed. Synthetic Peptides[20]. Hence, peptides can be synthesized using the automated Merrifield techniques of solid phase synthesis with the α-NH$_2$ protected by either t-Boc or F-moc chemistry using side chain protected amino acids on, for example, an Applied Biosystems Peptide Synthesizer Model 430A or 431.

When A is a fatty acid, it can be easily added to the N-terminal amino group of the synthesized peptide by the well known dicyclohexyl-carbodiimide coupling method.

Pam$_3$Cys, lipoamino acid S-[2,3-Bis(palmitoyloxy)-(2R)-propyl-N-palmitoyl-(R)-cysteine may also be synthesized by chemical methods. Pam$_3$Cys can be coupled to, for example, the N terminus of the mIgE peptide by solid-phase synthesis using Pam$_3$Cys-OH in the final coupling step to link the lipoamino acid to a resin-bound mIgE peptide chain. To improve the solubility of the final coupled lipopeptide product, the solid-phase peptide can be elongated with additional serine and lysine residues at the N-terminus.

After complete assembly of the desired immunogen, the resin is treated according to standard procedures to cleave the peptide from the resin and deblock the functional groups on the amino acid side chains. The free peptide is purified by HPLC and characterized biochemically, for example, by amino acid analysis or by sequencing. Purification and characterization methods for peptides are well known to one of ordinary skill in the art.

Other chemical means to generate the linear peptide constructs of the invention containing mIgE and Th sites include the ligation of the haloacetylated and the cysteinylated peptide through the formation of a

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"thioether" linkage. For example, a cysteine can be added
to the C terminus of a Th-containing peptideand the thiol
group of cysteine may be used to form a covalent bond to
an electrophilic group such as an N^{α} chloroacetyl-modified
or a maleimide-derivatized α- or ε-NH_2 group of a lysine
residue attached to the N-terminus of an mIgE membrane
anchor peptide (SEQ ID NOS:1 or 2) or immunogenic analogs
thereof. In this manner, a construct with Th-(mIgE
peptide) or its reverse, (mIgE peptide)-Th, may be
obtained.

The subject immunogen may also be polymerized.
Polymerization can be accomplished for example by reaction
between glutaraldehyde and the -NH_2 groups of the lysine
residues using routine methodology. By another method,
the linear "A-Th-spacer-(mIgE peptide)" or "(mIgE
peptide)-spacer-$(Th)_m$-$(A)_n$" immunogen can be polymerized
or co-polymerized by utilization of an additional cysteine
added to the N-terminus of the linear "A-Th-spacer-(mIgE
peptide) or "(mIgE peptide)-spacer-$(Th)_m$-$(A)_n$" immunogen.
The thiol group of the N-terminal cysteine can be used for
the formation of a "thioether" bond with haloacetyl-
modified amino acid or a maleimide-derivatized α- or ε-NH_2
group of a lysine residue that is attached to the N-
terminus of a branched poly-lysyl core molecule (e.g.,
K_2K, K_4K_2K or $K_8K_4K_2K$).

Alternatively, the longer linear peptide
immunogens can be synthesized by well known recombinant
DNA techniques. Any standard manual on DNA technology
provides detailed protocols to produce the peptides of the
invention. To construct a gene encoding a peptide of this
invention, the amino acid sequence is reverse translated
into a nucleic acid sequence, and preferably using
optimized codon usage for the organism in which the gene
will be expressed. Next, a synthetic gene is made,
typically by synthesizing overlapping oligonucleotides
which encode the peptide and any regulatory elements, if

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n cessary. The synthetic gene is inserted in a suitable
cloning vector and recombinants are obtained and
characterized. The peptide is then expressed under
suitable conditions appropriate for the selected
expression system and host. The peptide is purified and
5   characterized by standard methods.

The efficacy of the immunogen of the present
invention can be established by injecting an animal, for
example, rats, followed by monitoring the humoral immune
response to the mIgE membrane anchor peptides (SEQ ID
10   NOS:1,2) and immunogenic analogs thereof, as detailed in
the Examples.

Another aspect of this invention provides a
vaccine composition comprising an immunologically
effective amount of one or more of the immunogens of this
15   invention in a pharmaceutically acceptable delivery
system. Such vaccine compositions are used for prevention
of atopic allergic reactions including allergic rhinitis,
those of food allergies, asthma, anaphylaxis, and other
IgE-mediated hypersensitive reactions such as virally-
20   induced asthma.

Accordingly, the subject peptides can be
formulated as a vaccine composition using adjuvants,
pharmaceutically-acceptable carriers or other ingredients
routinely provided in vaccine compositions. Such
25   formulations are readily determined by one of ordinary
skill in the art and include formulations for immediate
release and/or for sustained release, and for induction of
systemic immunity and/or induction of localized mucosal
immunity, which may be accomplished by, for example,
30   immunogen entrapment by microparticles. The present
vaccines can be administered by any convenient route
including subcutaneous, oral, intramuscular, or other
parenteral or enteral route. Similarly the vaccines can
be administered as a single dose or multiple doses.
35   Immunization schedules are readily determined by the

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ordinarily skilled artisan. Adjuvants or emulsifiers that
can be used in this invention include alum, incomplete
Freund's adjuvant, liposyn, saponin, squalene, L121,
emulsigen and ISA 720 as well as the other efficacious
adjuvants and emulsifiers.

The vaccine composition of the instant invention
contain an effective amount of one or more of the
immunogens of the present invention and a pharmaceutically
acceptable carrier. Such a composition in a suitable
dosage unit form generally contains about 0.5 μg to about
1 mg of the immunogen per kg body weight. When delivered
in multiple doses, it may be conveniently divided into an
appropriate amount per dosage unit form.

Vaccines which contain cocktails of the subject
immunogens with two or more of the Th epitopes may enhance
immunoefficacy in a broader population and thus provide an
improved immune response to the mIgE membrane anchor
peptide (SEQ ID NOS:1,2). For example, a cocktail of
Peptide Nos. 1-4 of Example 1 and 7-10 of Example 4 is
useful. Other immune stimulatory synthetic peptide-based
mIgE anchor peptide immunogens are arrived at through
modification into lipopeptides, such as Pam_3Cys, so as to
provide a built-in adjuvant for a potent vaccine. The
immune response to synthetic mIgE anchor peptide-
containing immunogens can be improved by delivery through
entrapment in or on biodegradable microparticles of the
type described by O'Hagan et al[21]. The immunogens can be
encapsulated with or without an adjuvant, including
covalently attached Pam_3Cys, and such microparticles can
carry an immune stimulatory adjuvant such as Freund's
Incomplete Adjuvant or alum. The microparticles function
to potentiate immune responses to an immunogen, including
localized mucosal immunity which may be especially
applicable to mucosally localized allergic reactions, and
to provide time-controlled release for sustained or

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periodic responses, for oral administration, and for
topical administration[21,22].

    Specific peptide immunogens and compositions are
provided in the following examples to illustrate the
invention. The peptide immunogen of the invention can be
useful for the amelioration of IgE-mediated allergic
disorders.

EXAMPLE 1

SYNTHESIS OF PEPTIDE IMMUNOGENS NOS 1-4 "Th-SPACER-(mIgE PEPTIDE)" AND "(mIgE PEPTIDE)-SPACER-Th"

Immunogens 1-4 (Table III) were synthesized by solid phase
synthesis using F-moc chemistry on an Applied Biosystems
Peptide Synthesizer Model 430A or 431 according to
manufacturer's instructions. After complete assembly of
the peptide, the resin was treated according to standard
procedures to cleave the peptide from the resin and
deprotect the functional groups on amino acid side chains.
The free peptide was then purified by HPLC and
characterized biochemically for amino acid content and
sequence.

    The structures of Peptide immunogens Nos. 1-4,
from the amino terminus to the carboxyl terminus, are
symbolized as A-Th-B-(mIgE peptide) or (mIgE peptide)-B-
Th-A, where "A" is NH_2-, "B" is a Gly-Gly spacer, "Th" is
the measles virus F1 helper T cell epitope MV_{F1} Th (SEQ ID
NO:12), and "(mIgE peptide)" is either mIgE peptide of SEQ
ID NO:1 or mIgE peptide of SEQ ID NO:2. Thus, Peptide No.
1 may be more explicitly represented as "MV_{F1} Th-GG-(mIgE
1)" and Peptide No. 2 as "(mIgE 2)-GG-MV_{F1} Th", for
examples. The actual sequences for Peptides 1-4 (SEQ ID
NOS:25-28) are shown in Table 2.

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TABLE 2

Amino Acid Sequences of Peptide Nos. 1-4

Peptide	Sequence
1. MV$_{Fi}$Th-GG-mIgE1	Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val-Gly-Gly-Leu-Ala-Gly-Gly-Ser-Ala-Gln-Ser-Gln-Arg-Ala-Pro-Asp-Arg-Val-Leu-Cys-His-Ser-Gly-Gln-Gln-Gln-Gly-Leu (SEQ ID No:25)
2. mIgE1-GG-MV$_{Fi}$Th	Leu-Ala-Gly-Gly-Ser-Ala-Gln-Ser-Gln-Arg-Ala-Pro-Asp-Arg-Val-Leu-Cys-His-Ser-Gly-Gln-Gln-Gln-Gly-Leu-Gly-Gly-Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val (SEQ ID No:26)
3. MV$_{Fi}$Th-GG-mIgE2S	Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val-Gly-Gly-Pro-Glu-Leu-Asp-Val-Cys-Val-Glu-Glu-Ala-Glu-Gly-Glu-Ala-Pro-Trp-Thr (SEQ ID No:27)
4. mIgE2-GG-MV$_{Fi}$Th	Pro-Glu-Leu-Asp-Val-Cys-Val-Glu-Glu-Ala-Glu-Gly-Glu-Ala-Pro-Trp-Thr-Gly-Gly-Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val (SEQ ID No:28)

EXAMPLE 2

IMMUNIZATION OF RATS WITH "Th-SPACER-(mIgE PEPTIDE)" AND "(mIgE PEPTIDE)-SPACER-Th" LINEAR CONSTRUCTS, PEPTIDE NOS. 1-4, AND EVALUATION OF IMMUNOGENICITY BY ELISA

Efficacy of Peptide immunogens Nos. 1-4 is evaluated on groups of five rats by the experimental immunization protocol outlined below and by serological assay for d termination of immunogenicity.

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Experimental Design:

>   Immunogen: Peptide Nos. 1-4 (1 per group)
>   Dose: 100 µg per immunization
>   Route: intramuscular
>   Adjuvant: Freund's Complete/Incomplete

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>   Dose Schedule: week 0 (FCA), 3 and 6 weeks

(IFA)

>   Bleed Schedule: weeks 0, 3, 6, 8, 10
>   Species: Sprague-Dawley rats
>   Group size: 5

10

>   Assay: ELISAs for anti-peptide activity.

Blood is collected and processed into serum, and
stored prior to titering by ELISA with the target peptides
(SEQ ID NOS:1,2).

Anti-peptide antibody activities are determined

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by ELISA (enzyme-linked immunosorbent assay) using 96-well
flat bottom microtiter plates which are coated with the
corresponding target mIgE peptide epitope as the
immunosorbent, either Peptide 5 for mIgE anchor peptide
site described by SEQ ID NO:1 or Peptide No. 6, described

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by SEQ ID NO:2.   Aliquots (100 µL) of a peptide immunogen
solution at a concentration of 5 µg/mL are incubated for 1
hour at 37°C.   The plates are blocked by another incubation
at 37°C for 1 hour with a 3% gelatin/PBS solution.   The
blocked plates are then dried and used for the assay.

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Aliquots (100 µL) of the test rat sera, starting with a
1:10 dilution in a sample dilution buffer and ten-fold
serial dilutions thereafter, are added to the peptide
coated plates.   The plates are incubated for 1 hour at
37°C.

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The plates are washed six times with 0.05%
PBS/Tween® buffer.   100 µL of horseradish peroxidase
labelled goat-anti-rat antibody is added at a dilution of
1:1,000 in conjugate dilution buffer (Phosphate buffer
containing 0.5M NaCl, and normal goat serum).   The plates

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are incubated for 1 hour at 37°C before being washed as

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above. Aliquots (100 μL) of o-phenylenediamine substrate
solution are then added. The color is allowed to develop
for 5-15 minutes before the enzymatic color reaction is
stopped by the addition of 50 μL 2N H_2SO_4. The A_{492nm} of the
contents of each well is read in a plate reader.

All sera are assayed by anti-peptide ELISA and
those samples which give A_{492nm} values of ≥ 0.2 at a 1:100
dilution are recorded as seropositive. Normal rat serum
is used as a negative control. Results are also compared
to positive control serum from rats immunized with KLH
conjugates of either Peptide No. 5 or 6 described above, to
demonstrate improved immunogenicity for peptide of the
invention.

EXAMPLE 3
EVALUATION OF RAT ANTI-SERA TO PEPTIDE NOS. 1-4 FOR
FUNCTIONAL EFFICACY ON HUMAN IgE-SECRETING CELL LINES

The peptide immunogen compositions of this
invention evoke antibodies that target IgE-secreting human
B cells and inhibit IgE production. Unlike most antibodies
to IgE, the mIgE peptide-elicited antibodies do not bind to
cells bearing only the secretory form of IgE bound to
receptors and are therefore incapable, by themselves, of
triggering the release of the chemical mediators of
allergic response from IgE-sensitized mast cells and
basophils. These biological activities are of relevance to
immunotherapy for allergy and can be observed in the rat
antisera to Peptide immunogens Nos. 1-4 by assaying for the
following functions:

1. <u>Antibody binding to IgE-secreting B cells and not
binding to IgE-sensitized basophils.</u> A human IgE-
producing B cell line, for example the myeloma cell line
SKO-007 (ATCC, Rockville MD) or an EBV-transformed B cell
line 8866, is incubated with serial dilutions of the rat
anti-mIgE and antibody binding is detected using FITC-

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labeled ant-rat IgG and quantitated by fluorescence flow
cytometry[4]. The extent of binding by the rat anti-mIgE
antibodies to normal human basophils, prepared from
peripheral blood and loaded or sensitized with secreted
IgE, is evaluated in a similar fashion[5].

2. <u>Reduction in the IgE accumulation of IgE-secreting
cells.</u> IgE accumulates in the culture medium of myeloma
cell line SKO-007 and in like IgE-secreting cell lines.
To determine whether treatment of IgE-secreting cells with
the rat anti-mIgE sera elicited by Peptide immunogens Nos.
1-4 results in a decrease of IgE secreted into the medium,
the cells are treated with a range of antibody
concentrations, and IgE levels in the medium are monitored
over time by IgE-specific ELISA[11]. Efficacious antibodies
result in a dose-related reduction in accumulated IgE.

3. <u>Lysis of IgE-secreting cells by antibody dependent
cell-mediated cytotoxicity (ADCC).</u> The capability of a
range of concentrations of the rat anti-mIgE sera to induce
cytolysis of SKO-007 cells, or the like, by ADCC activity
is evaluated for percent lysis by an assay employing
effector cells from several donors[11].

4. <u>Inability to induce histamine release from basophils.</u>
Rat anti-mIgE sera is used at several concentrations to
show a dose-related ability to induce histamine release
from IgE-sensitized isolated peripheral blood basophils.
Histamine-release is quantitated by fluorometric assay[5].
An inability to induce histamine release is evidence for
the efficacy and safety of the peptide immunogen of the
present invention.

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EXAMPLE 4

COCKTAIL OF PEPTIDE IMMUNOGENS
TO FURTHER BROADEN THE RESPONSIVE POPULATION

A cocktail of immunogenic Th-spacer-(mIgE
peptide) and (mIgE peptide)-spacer-Th peptides wherein
there are more than one Th epitope for recognition by
diverse MHC types can serve to broaden immune
responsiveness in a genetically diverse human population.
Promiscuous Th epitopes useful for this purpose are
selected from Table II. The Th epitopes of Table II that
are useful for such peptide cocktails include but are not
limited to the MV_{F1} Th peptide (SEQ ID NO:12) used in
Peptide Nos. 1-4 (SEQ ID NOS:25-28) and the HB_s Th peptide
(SEQ ID NO:5). Peptides containing either of the two
anchor membrane mIgE peptide sequences (SEQ ID NOS:1 or 2)
and the HB_s Th peptide are described in Table IV as Peptide
immunogens Nos. 7-10 (SEQ ID NOS: 29-32).

Peptide immunogens Nos. 7-10 are synthesized as
described in Example 1 and combined with each other and
with Peptide immunogens Nos. 1-4, in equal molar ratios, to
formulate into a peptide cocktail. A composition of the
invention formulated as a cocktail is evaluated for
immunopotency in rats by the protocol described below.

Experimental Design:

Immunogens:	(1) Cocktail : Peptide Nos. 1-4 and 7-10	
	(2) Positive Control: Individual KLH conjugates of Peptide Nos. 5 and 6 combined 1:1 (One immunogen per group of rats)	
Dose:	Molar equivalents of each synthetic peptide immunogen or mIgE peptide equivalent, to equal 100 μg total or 12.5 μg of each peptide per immunization	

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Route:	intramuscular
Adjuvants:	(1) Freund's Complete/Incomplete
	(2) 0.4% Alum (Aluminum hydroxide)
	(One of either adjuvant per
	immunogen per group).
Dose Schedule:	week 0, 2 and 4 weeks
	CFA/IFA groups receive CFA week 0,
	IFA weeks 2 and 4. Alum groups
	receive Alum formulations for all
	3 doses)
Bleed Schedule:	weeks 0, 3, 6 and 8
Species:	Sprague-Dawley rats
Group size:	5, 4 groups
Assay:	2 ELISAs for anti-peptide
	activity, solid-phase substrates
	are Peptide Nos. 5 and 6 (SEQ ID
	NOS:1,2)

Blood is collected, processed into serum and stored prior for determination of seroconversion by the two anti-peptide ELISAs as described in Example 2.

This study is designed to demonstrate improved immunogenicity for this embodiment of the peptide invention, and to demonstrate efficacy for a composition of the invention formulated with a pharmaceutically acceptable adjuvant, Alum.

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TABLE IV

Amino Acid Sequences of Peptide Nos. 7-10

Peptide	Sequence
7. HB,Th-GG-mIgE1	Phe-Phe-Leu-Leu-Thr-Arg-Ile-Leu- Thr-Ile-Pro-Gln-Ser-Leu-Asp-Gly- Gly-Leu-Ala-Gly-Gly-Ser-Ala-Gln- Ser-Gln-Arg-Ala-Pro-Asp-Arg-Val- Leu-Cys-His-Ser-Gly-Gln-Gln-Gln- Gly-Leu (SEQ ID No:29)
8. mIgE1-GG-HB,Th	Leu-Ala-Gly-Gly-Ser-Ala-Gln-Ser- Gln-Arg-Ala-Pro-Asp-Arg-Val-Leu- Cys-His-Ser-Gly-Gln-Gln-Gln-Gly- Leu-Gly-Gly-Phe-Phe-Leu-Leu-Thr- Arg-Ile-Leu-Thr-Ile-Pro-Gln-Ser- Leu-Asp (SEQ ID No:30)
9. HB,Th-GG-mIgE2	Phe-Phe-Leu-Leu-Thr-Arg-Ile-Leu- Thr-Ile-Pro-Gln-Ser-Leu-Asp-Gly- Gly-Pro-Glu-Leu-Asp-Val-Cys-Val- Glu-Glu-Ala-Glu-Gly-Glu-Ala-Pro- Trp-Thr (SEQ ID No:31)
10. mIgE2-GG-HB,Th	Pro-Glu-Leu-Asp-Val-Cys-Val-Glu- Glu-Ala-Glu-Gly-Glu-Ala-Pro-Trp- Thr-Gly-Gly-Phe-Phe-Leu-Leu-Thr- Arg-Ile-Leu-Thr-Ile-Pro-Gln-Ser- Leu-Asp (SEQ ID No:32)

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EXAMPLE 5

CLINICAL TRIAL TO DEMONSTRATE
THERAPEUTIC EFFICACY OF COCKTAIL

Individual constructs carrying Th peptide sequences from measles virus F and hepatitis B surface antigen are promiscuous for multiple human HLA DR antigens, so as to provide maximum immunogenicity in a genetically diverse human population. Moreover, because these Th peptides are derived from children's vaccines, childhood vaccinations are a potential source of Th memory in an immunized human population. Thus, children's vaccines have the potential to afford enhanced immunopotency to anti-allergy vaccines comprised of mixtures of such Th peptides. The clinical protocol below is designed to demonstrate efficacy for compositions of the invention formulated as a mixture of such linear "Th-Spacer-(mIgE Peptide)" and "(mIgE Peptide)-spacer-Th" peptide constructs, in a widely acceptable adjuvant, Alum.

Experimental Design:

Subjects:	Hay fever patients
Season & Duration:	Hay fever seasons, 8 weeks
Groups:	4 groups, 1 group/immunogen/dose
	N=15 per group,
	12 receive immunogen, 3 receive
	placebo
Immunogen:	Cocktail 1; Peptide Nos. 1-4, 7-10
Adjuvant:	0.2% Alum
Dose:	Molar equivalents of each synthetic
	peptide to equal 500 μg total or
	62.5 μg of each peptide per dose
Route:	intramuscular
Dose Schedule:	week 0, and 4 weeks
Evaluation schedule:	weeks 0, 4, and 6

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Blood is collected, processed into serum, and stored prior
to titering by ELISA as described in Example 2.

Efficacy and safety of the clinical composition
are evaluated by comparisons of serological tests, skin test
reaction, by recording patient usage of hay fever

5 medication, by physical examination for allergic symptoms
and adverse reactions, and by interviews to obtain
subjective patient assessments of the product. Serological
evaluations include the aforementioned ELISAs for
antipeptide titers, and a standard automated

10 spectrofluorimetric assay to determine reduction in
histamine levels[13] as well as to ascertain that the products
do not trigger histamine release. The skin test is an
intradermal test in which a standardized solution of
allergens is injected into the upper layers of the skin.

15 Reactions to the allergens are quantitated in the skin test
by determining the area of the typical "wheal and flare"
produced in response to the allergens. The expected results
include significant improvement in allergic symptoms at the
endpoint of the study, and no evidence of histamine release.

20 This experiment demonstrates the clinical efficacy
and safety of a pharmaceutically acceptable composition of
the invention.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: United Biomedical Inc.;
              Walfield, Alan M.; Wang, Chang Yi

   (ii) TITLE OF INVENTION: Synthetic IgE Membrane Anchor
        Peptide Immunogens for the Treatment of Allergy

  (iii) NUMBER OF SEQUENCES: 32

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    (v) COMPUTER READABLE FORM:
       (A) MEDIUM TYPE: Floppy disk
       (B) COMPUTER: IBM PC compatible
       (C) OPERATING SYSTEM: PC-DOS/MS-DOS
       (D) SOFTWARE: WordPerfect 5.1

   (vi) PRIOR APPLICATION DATA:
       (A) APPLICATION NUMBER: 08/328,519
       (B) FILING DATE: 25-OCT-1994
       (C) CLASSIFICATION:

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       (A) APPLICATION NUMBER: TO BE ASSIGNED
       (B) FILING DATE: 25-OCT-1995
       (C) CLASSIFICATION:

 (viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 26 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Gly Leu Ala Gly Gly Ser Ala Gln Ser Gln Arg Ala Pro
 1   5   10

Asp Arg Val Leu Cys His Ser Gly Gln Gln Gln Gly Leu
    15   20   25

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 17 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

    Pro Glu Leu Asp Val Cys Val Glu Glu Ala Glu Gly Glu
     1   5   10

    Ala Pro Trp Thr
        15

20 (2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 16 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

    Thr Ala Lys Ser Lys Lys Phe Pro Ser Tyr Thr Ala Thr
    1   5   10

    Tyr Gln Phe
        15

    (2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 6 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

```
       (ii) MOLECULE TYPE: peptide

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

        Pro Pro Xaa Pro Xaa Pro
        1               5

(2) INFORMATION FOR SEQ ID NO:5:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 15 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: peptide

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

        Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln
        1               5                   10

        Ser Leu Asp
                15


(2) INFORMATION FOR SEQ ID NO:6:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 30 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: peptide

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

        Lys Lys Leu Arg Arg Leu Leu Tyr Met Ile Tyr Met Ser
          1               5              10

        Gly Leu Ala Val Arg Val His Val Ser Lys Glu Glu Gln
            15                  20                  25

        Tyr Tyr Asp Tyr
                30


(2) INFORMATION FOR SEQ ID NO:7:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 17 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear
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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Lys Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly
 1   5   10

Ile Thr Glu Leu
     15

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 22 amino acids
         (B) TYPE: amino acid
         (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Lys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg
 1   5   10

Val Pro Lys Val Ser Ala Ser His Leu
         15   20

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 15 amino acids
         (B) TYPE: amino acid
         (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Tyr Met Ser Gly Leu Ala Val Arg Val His Val Ser Lys
 1   5   10

Glu Glu
     15

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 27 amino acids
         (B) TYPE: amino acid
         (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Tyr Asp Pro Asn Tyr Leu Arg Thr Asp Ser Asp Lys Asp
 1   5   10

Arg Phe Leu Gln Thr Met Val Lys Leu Phe Asn Arg Ile
     15   20   25

Lys

(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 24 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly Ala Tyr Ala Arg Cys Pro Asn Gly Thr Arg Ala Leu
 1   5   10

Thr Val Ala Glu Leu Arg Gly Asn Ala Glu Leu
     15   20

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 15 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Leu Ser Glu Ile Lys Gly Val Ile Val His Arg Leu Glu
 1   5   10

Gly Val
     15

(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 21 amino acids

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    (B) TYPE: amino acid
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Val Ser Phe Gly Val Trp Ile Arg Thr Pro Pro Ala Tyr
1   5   10

Arg Pro Pro Asn Ala Pro Ile Leu
    15   20

(2) INFORMATION FOR SEQ ID NO:14:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gly Ile Leu Glu Ser Arg Gly Ile Lys Ala Arg Ile Thr
1   5   10

His Val Asp Thr Glu Ser Tyr
    15   20

(2) INFORMATION FOR SEQ ID NO:15:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 17 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Trp Val Arg Asp Ile Ile Asp Asp Phe Thr Asn Glu Ser
1   5   10

Ser Gln Lys Thr
    15

(2) INFORMATION FOR SEQ ID NO:16:

    (i) SEQUENCE CHARACTERISTICS:

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       (A) LENGTH: 16 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: peptide

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

    Asp Val Ser Thr Ile Val Pro Tyr Ile Gly Pro Ala Leu
     1   5   10

    Asn Ile Val
         15

(2) INFORMATION FOR SEQ ID NO:17:

     (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 25 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: peptide

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

    Ala Leu Asn Ile Trp Asp Arg Phe Asp Val Phe Cys Thr
     1   5   10

    Leu Gly Ala Thr Thr Gly Tyr Leu Lys Gly Asn Ser
        15   20   25

(2) INFORMATION FOR SEQ ID NO:18:

     (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 23 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: peptide

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

    Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys Thr Val Ala
     1   5   10

    Ala Leu Ser Ile Leu Pro Gly Ile Gly Cys
        15   20

(2) INFORMATION FOR SEQ ID NO:19:

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    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 39 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Glu Glu Ile Val Ala Gln Ser Ile Ala Leu Ser Ser Leu
 1               5                  10

Met Val Ala Gln Ala Ile Pro Leu Val Gly Glu Leu Val
        15                  20                  25

Asp Ile Gly Phe Ala Ala Thr Asn Phe Val Glu Ser Cys
            30                  35
```

(2) INFORMATION FOR SEQ ID NO:20:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 21 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Asp Ile Glu Lys Lys Ile Ala Lys Met Glu Lys Ala Ser
 1               5                  10

Ser Val Phe Asn Val Val Asn Ser
        15                  20
```

(2) INFORMATION FOR SEQ ID NO:21:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 17 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Lys Trp Phe Lys Thr Asn Ala Pro Asn Gly Val Asp Glu
 1               5                  10

Lys Ile Arg Ile
    15
```

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(2) INFORMATION FOR SEQ ID NO:22:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 14 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

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   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

   Gly Leu Gln Gly Lys His Ala Asp Ala Val Lys Ala Lys
   1   5   10

10   Gly

(2) INFORMATION FOR SEQ ID NO:23:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 19 amino acids
15   (B) TYPE: amino acid
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

   Gly Leu Ala Ala Gly Leu Val Gly Met Ala Ala Asp Ala
20   1   5   10

   Met Val Glu Asp Val Asn
      12

(2) INFORMATION FOR SEQ ID NO:24:

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    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 20 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

30   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

   Ser Thr Glu Thr Gly Asn Gln His His Tyr Gln Thr Arg
   1   5   10

   Val Val Ser Asn Ala Asn Lys
      15   20

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(2) INFORMATION FOR SEQ ID NO:25:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 42 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Leu Ser Glu Ile Lys Gly Val Ile Val His Arg Leu Glu
 1   5   10

Gly Val Gly Gly Leu Ala Gly Gly Ser Ala Gln Ser Gln
   15   20   25

Arg Ala Pro Asp Arg Val Leu Cys His Ser Gly Gln Gln
        30   35

Gln Gly Leu
  40

(2) INFORMATION FOR SEQ ID NO:26:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 43 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Gly Leu Ala Gly Gly Ser Ala Gln Ser Gln Arg Ala Pro
 1   5   10

Asp Arg Val Leu Cys His Ser Gly Gln Gln Gln Gly Leu
   15   20   25

Gly Gly Leu Ser Glu Ile Lys Gly Val Ile Val His Arg
        30   35

Leu Glu Gly Val
  40

(2) INFORMATION FOR SEQ ID NO:27:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 34 amino acids
       (B) TYPE: amino acid

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            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

        Leu Ser Glu Ile Lys Gly Val Ile Val His Arg Leu Glu
         1               5                   10

        Gly Val Gly Gly Pro Glu Leu Asp Val Cys Val Glu Glu
            15                  20                  25

        Ala Glu Gly Glu Ala Pro Trp Thr
                        30


    (2) INFORMATION FOR SEQ ID NO:28:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 34 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

        Pro Gly Leu Asp Val Cys Val Glu Glu Ala Glu Gly Glu
         1               5                   10

        Ala Pro Trp Thr Gly Gly Leu Ser Glu Ile Lys Gly Val
            15                  20                  25

        Ile Val His Arg Leu Glu Gly Val
                        30


    (2) INFORMATION FOR SEQ ID NO:29:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 42 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

        Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser
         1               5                   10

        Leu Asp Gly Gly Leu Ala Gly Gly Ser Ala Gln Ser Gln
            15                  20                  25
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            Arg Ala Pro Asp Arg Val Leu Cys His Ser Gly Gln Gln
                    30   35

            Gln Gly Leu
             40

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    (2) INFORMATION FOR SEQ ID NO:30:

            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 43 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear

10   (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

            Gly Leu Ala Gly Gly Ser Ala Gln Ser Gln Arg Ala Pro
             1   5   10

15   Asp Arg Val Leu Cys His Ser Gly Gln Gln Gln Gly Leu
             15   20   25

            Gly Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro
                    30   35

            Gln Ser Leu Asp
             40
20

    (2) INFORMATION FOR SEQ ID NO:31:

            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 34 amino acids
                (B) TYPE: amino acid
25   (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

            Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser
             1   5   10
30
            Leu Asp Gly Gly Pro Glu Leu Asp Val Cys Val Glu Glu
                    15   20   25

            Ala Glu Gly Glu Ala Pro Trp Thr
                         30

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(2) INFORMATION FOR SEQ ID NO:32:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 34 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Pro Glu Leu Asp Val Cys Val Glu Glu Ala Glu Gly Glu
 1           5                   10

Ala Pro Trp Thr Gly Gly Phe Phe Leu Leu Thr Arg Ile
     15              20                  25

Leu Thr Ile Pro Gln Ser Leu Asp
             30
```

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We claim:

1.   A peptide immunogen selected from the group
consisting of:

$$(A)_n-(Th)_m-(B)_o-(mIgE\ peptide)$$

or

$$(mIgE\ peptide)-(B)_o-(Th)_m-(A)_n$$

wherein: A is an amino acid, α-NH_2, a fatty acid or a
derivative thereof, or an invasin domain having
immmune stimulatory property;

B is an amino acid;

Th is a helper T cell epitope, or an immune
enhancing analog or segment thereof;

mIgE peptide is SEQ ID NO:1, SEQ ID NO:2, or
an immunogenic analog thereof;

n is from 1 to about 10;

m is from 1 to about 4; and

o is from 0 to about 10.

2.   The peptide immunogen of Claim 1 wherein said
Th has an amino acid sequence selected from the group of
sequences selected from SEQ ID NOS:5-24 or an immunogenic
analog or segment thereof.

3.   The peptide immunogen of Claim 1 having an
amino acid sequence selected from the group consisting of:
SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:27, SEQ
ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ
ID NO:32.

4.   The peptide immunogen of Claim 2 further
comprising a fatty acid.

5.   The peptide immunogen of Claim 2 further
comprising a fatty acid derivative.

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6.   The peptide immunogen of Claim 5 wherein the fatty acid derivative is Pam₃Cys.

7.   The peptide immunogen of Claim 3 further comprising a fatty acid.

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8.   The peptide immunogen of Claim 3 further comprising a fatty acid derivative.

9.   The peptide immunogen of Claim 8 wherein the
10 fatty acid derivative is Pam₃Cys.

10.   A vaccine composition comprising an effective amount of a peptide immunogen of Claim 1 and a pharmaceutically acceptable delivery system.

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11.   A vaccine composition comprising an effective amount of a peptide immunogen of Claim 2 and a pharmaceutically acceptable delivery system.

20   12.   A vaccine composition comprising an effective amount of a peptide immunogen of Claim 3 and a pharmaceutically acceptable delivery system.

13.   A vaccine composition comprising an effective
25 amount of a peptide immunogen of Claim 4 and a pharmaceutically acceptable delivery system.

14.   A vaccine composition comprising an effective amount of a peptide immunogen of Claim 5 and a
30 pharmaceutically acceptable delivery system.

15.   A vaccine composition comprising an effective amount of a peptide immunogen of Claim 6 and a pharmaceutically acceptable delivery system.

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16. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 7 and a pharmaceutically acceptable delivery system.

17. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 8 and a pharmaceutically acceptable delivery system.

18. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 9 and a pharmaceutically acceptable delivery system.

19. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 1.

20. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 2.

21. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 3.

22. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 4.

23. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 5.

24. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 6.

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25. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 7.

26. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 8.

27. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 9.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/13841

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :CO7K 16/100, 16/46; A61K 39/00, 39/35
US.CL : 530/324, 387.1, 403; 424/184.1, 185.1; 514/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 387.1, 403; 424/184.1, 185.1; 514/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS & CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,260,416 (CHANG) 09 NOVEMBER 1993, see entire document.	1-27
Y	US, A, 5,231,026 (CHANG) 27 JULY 1993, see entire document.	1-27
Y	US, A, 5,281,699 (CHANG) 25 JANUARY 1994, see entire document.	1-27
Y	US, A, 5,274,075 (CHANG) 28 DECEMBER 1993, see entire document.	1, 9, 19
Y	US, A, 5,342,924 (CHANG) 30 AUGUST 1994, see entire document.	1, 9, 19

| X | Further documents are listed in the continuation of Box C. | | See patent family annex. |

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 FEBRUARY 1996	05 MAR 1996

Name and mailing address of the ISA/US	Authorized officer
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	THE LANCET, Volume 336, issued 1990, Stanworth et al, "Allergy Treatment with a peptide vaccine", pages 1279-1281, see entire document.	1-27
A	The Journal of Immunology, volume 148, number 1, issued 01 January 1992, Peng et al, "A new isoform of human membrane bound IgE," pages 129-136, see entire document.	1-27
Y	Molecular Immunology volume 23, number 1, issued 1986, Stanworth et al, "Anti - e - chain antibodies as probes in the study of mast-cell triggering", pages 1231-1235, see entire document.	1-27
Y	European J. Immunology, volume 17, issued 1987, Leclerc et al, "A synthetic vaccine constructed by copolymerization of B and T cell determinants", pages 269-273, see entire document.	1-27
Y	US, A, 4,722,840 (VALENZUELA ET AL) 02 February 1988, see entire document.	1-27